The trends of the larger folds have been plotted in Text-figure 1, where it can be clearly seen that the major axis of the anticlinorium is inclined to that of the Catombal Syncline. As the syncline, which is occupied by rocks of Lower, Middle and Upper Devonian age, transgresses the larger fold, the latter must have formed in pre-Devonian time and must be related to the Bowning Orogeny (Browne, 1947). The Catombal fold, on the other hand, is post-Devonian and probably formed during the Kanimbla Orogeny. Although it is impossible to interpret the structure when the configuration and nature of the basement are unknown, reference to Text-figure 1 will show that the Cudal Anticline, the Borenore Syncline and the small basin south of the Molong Dome radiate inwards towards the southern end of the Catombal Syncline, and it is tentatively suggested that this gathering of the folds is due to the downwarping of the Catombal Syncline. It is also possible that the Barragan, Avenel and Cargo folds are also related, though their axes have been disturbed by subsequent faulting. The Molong Dome and Copper Hill Anticline also trend towards the Catombal axis and may have been caused by the same movement. A gathering of the folds at the northern end of the axis is not so apparent although the Poggy Fault, and a small syncline about one mile east of it, show such a trend. The Maryvale Dome is possibly on the main axis of the Silurian fold and the Ponto and Suntop Anticlines, as well as a small anticline north of the Narragal Fault, are nearly parallel to the Bowning axis of folding and are possibly related to it.

Faults.

Basnett and Colditz recorded four faults in the Wellington area and Stevens mapped two major faults in the southern area. With the possible exception of the Poggy Fault all appear to be pre-Devonian, and to have transgressed probable Kanimbla folds, thus they may be late Kanimbla.

On account of the similarity between the serpentinized lamprophyre in the Poggy Fault, and the serpentine associated with augite-andesite at Lucknow, it is suggested that a fault occurs at this locality though it has not been mapped and the trend indicated is hypothetical. Detailed mapping will probably reveal other faults in the area, particularly in the region between Boree and Oakey Creeks, which has so far received little attention.

Unconformities.

In dealing with the stratigraphy it was indicated that two slight unconformities are shown—one between Silurian and Lower Devonian rocks and the other between rocks of Middle and Upper Devonian age (Brown, 1932).

Summary.

It has been indicated that the map is a compilation based on the work of sixteen people. Though some parts have been mapped in detail, it is mainly a piece of reconnaissance mapping. Nevertheless, it serves a useful purpose in giving a regional picture of the area and reveals slight unconformities between Silurian and Devonian rocks and between Middle and Upper Devonian strata. Furthermore, the general structure of the area is revealed, and it seems fairly evident that Kanimbla folding and faulting have been superimposed on an anticlinorium which was folded during the Bowning Orogeny.

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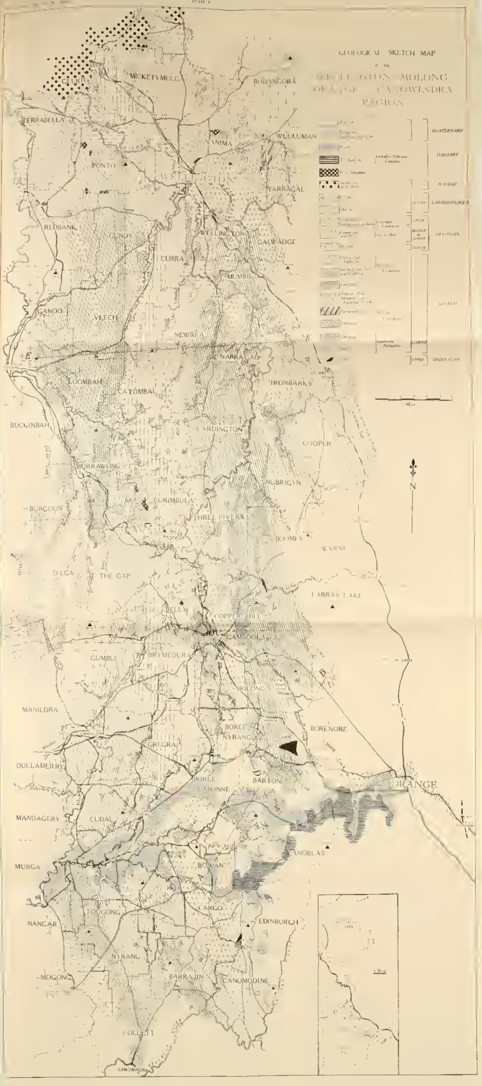
GEOLOGICAL SKETCH MAP
QUATERNARY
TERTIARY
JURASSIC
CARBONIFEROUS
DEVONIAN
ORDOVICIAN
LOWER
UPPER
N
MICKETYMULGA
BODANGORA
TERRABELLA
WUULUMAN
ANIMA
PONTO
WELLINGTON
YARRAGAL
REDBANK
GUNDY
GALWADGE
CURRA
MUMBIL
VEECH
NEWREA
NARRAGAL
LOOMBAH
CATOMBAL
IRONBARKS
BUCKINBAH
CARDINGTON
COOPER
BURRAWONG
NUBRIGYN
BURGOON
EURIMBULA
THREE RIVERS
BOOMEY
WARNE
THE GAP
LARRAS LAKE
BELL
COPPER HILL
GAMBOOLA
GUMBLE
BRYMEDURA
MOLONG
MANILDRA
BOREE
NYRANG
BORENORE
GREGRA
DULLADERRY
CABONNE
BARTON
ORANGE
MANDAGERY
CUDAL
CANOBLAS
MURGA
TOOGONG
CARGO
NANGAR
EDINBURGH
NYRANG
MOGONG
BARRAJIN
CANOMODINE
COLLETT
CANOWINDRA

STUDIES OF N-FIXING BACTERIA. I.

A Note on the Estimation of Azotobacter in the Soil.

By Y. T. Tchan, Macleay Bacteriologist to the Society.

[Read 30th April, 1952.]

Synopsis.

The controversy on the use of liquid and solid media for the estimation of Azotobacter is critically examined. Experiences show that both techniques have some advantages and difficulties. A combined technique is proposed.

In 1926 Winogradsky published an account of his silico-gel method for the estimation of *Azotobacter* in the soil. He mistrusted the old liquid medium and based his objections on the fact that, in a liquid medium, if *Clostridium* develops in the lower layers a certain amount of N can be fixed which destroys the electivity of the medium. If abundant CO_2 is given off the medium becomes unsuitable for the growth of *Azotobacter*. The presence of protozoa may also affect the test and prevent the formation of a characteristic pellicle on the surface of the liquid. Because of these difficulties, Winogradsky considered that a positive test for *Azotobacter* is possible only when a large number of Azotobacter cells is present.

Jensen (1940) first pointed out that the liquid medium is better for detecting the sporadic presence of *Azotobacter* in the soil, although he thought that the plate method could be as accurate as the liquid medium if a comparable amount of soil inoculum were used.

More recently McKnight (1949) confirmed Jensen's observation and considered that the liquid medium is more accurate than the plate method.

Derx (1950) considered Winogradsky's silico-gel method as a very efficient procedure for isolation of N-fixing organisms: "As will presently be seen, this method has led to an important deepening of our knowledge of N-fixing organisms."

For the study of free N-fixing organisms in soil, it seems to us important to make a choice between different techniques.

The difference between the plate method and the liquid medium is not only dependent on the physico-chemical constitution, but there is a big difference in the quantity of inoculum used. Apparently we cannot decide on the accuracy of each technique when the quantity of inoculum used is not the same: (0·02 gr. p. plate (10 cm. of diameter) and 1 gr. p. liquid medium).

Experiments were carried out to determine the accuracy of each technique.

Azotobacter chroococcum recently isolated from the soil (Sydney University) was used. This culture was first incubated at 30°C. for 24 hours. A small quantity of it was then inoculated on a glucose-Agar for 18 hours. A suspension of this young culture was made in sterilized distilled water. A microscopic examination shows that all cells were mobile and we may therefore assume that all the cells were living, although it is not safe to assume that they can all reproduce. A direct estimation with a haemocytometer gives the number of cells contained in 1 c.c. The suspension was then diluted successively, by a factor of 10, until the last dilution contained the required number of cells.

The medium used was Winogradsky's standard medium with 1·0% of glucose. For the plate count technique, 2% of washed Agar is added.

Five parallel sets of the two media were inoculated with 0·2 c.c. of each dilution, and incubated at 30°. The cultures were examined after one week; if no growth was then visible they were incubated for a further week.

Number of cells calculated/0·2 c.c. ..	200	20	2	0
Plate: Mean number of colonies (5 plates)	52	0	0	0
Liquid medium: Number of tubes positive	5	5	3	0

It is clear that the liquid medium is capable of giving a positive growth with a few Azotobacter cells, if not with a single cell. If soil were present, it is possible that one would not obtain such reliable results, as Winogradsky suggested.

In order to test this, a soil is needed which does not contain *Azotobacter*, but which has all the physico-chemical-biological qualities necessary for its growth. Fortunately, the Gilgai soil from Curlewis in New South Wales has the qualities required. A similar experiment carried out with 0·5 gr. of soil (5 parallel sets in each case).

Number of cells calculated/0·2 c.c.	1200	120	12	1·2	0
Plate count: Mean of 5 sets	Excess of colonies.	40·3	0	0	0
Liquid medium: Number of tubes positive ..	5	5	5	1	0
Liquid medium + soil: Number of tubes positive	5	5	5	3	0

In all tubes containing soil, there was an abundant development of *Clostridium*. A microscopic examination shows the presence also of protozoa which in our case do not affect the formation of a characteristic pellicle of *Azotobacter*.

This experiment seems to eliminate all Winogradsky's objections and the presence of soil seems favourable for the growth of *Azotobacter*.

In order to determine the effect of humus, an extract of humus was made from the Gilgai soil and added to the test solution. The addition of this humus to the medium did not seem to alter the growth of *Azotobacter* significantly.

Number of cells calculated	1000	100	10	1	0
Plate counts 5 sets	Excess of colonies	26	0	0	0
Liquid humus: Number of tubes positive ..	5	5	5	0	0
Liquid + soil: Number of tubes positive ..	5	5	5	1	0

Discussion.

In the tests carried out the liquid medium was more satisfactory than the 2% Agar medium for detecting the presence of *Azotobacter* in the soil. The Agar medium gives generally 25–33% of the theoretical number of colonies. Jensen (1940) found that the relationships between the plate count of *Azotobacter* and the corresponding direct counts of Azotobacter-like cells varied from 0·25% to 47%, with only 3 at 30–47% out of a total of 15 cases. Even if we accept that the Azotobacter-like cells in the soil are not all *Azotobacter*, our results agree quite well with Jensen's.

Using the statistical table given by McCrady (in Calmette et al., 1948) we can easily estimate the number of *Azotobacter* by the liquid medium.

Number of Cells Calculated	Plate Counts.	Liquid Count. Number Characteristic.	Number of *Azotobacter*.	Liquid Count + Soil. Number Characteristic.	Number of *Azotobacter*
200	52·0	553	90		
120	40·3	551	35	553	90
100	25·5	550 551	25 35	551	35

Although the liquid medium gave a better estimate of the total number of *Azotobacter* it does not give a proper estimate of the different species present.

Conclusion.

The above tests suggest that the liquid medium is more accurate than the 2% Agar plate for detecting the Azotobacter in soil. If we desire to have an idea of the different species composing the soil population (of *Azotobacter*) it is better to use the plate count technique.